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# Effect of Bromine Substitution on Human Dermal Absorption of Polybrominated Diphenyl Ethers

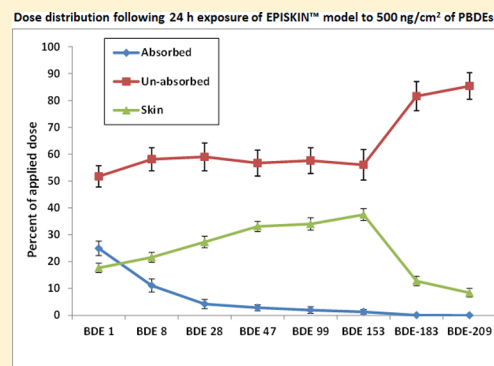
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## S Supporting Information

**ABSTRACT:** Human dermal absorption of eight mono- to deca-brominated diphenyl ethers (PBDEs) was investigated for the first time using EPISKIN human skin equivalent tissue. Using a standard *in vitro* protocol, EPISKIN tissues mounted in specially designed diffusion cells were exposed to the target PBDEs for 24 h. Estimated steady-state flux ( $J_{ss}$ ) and permeation coefficients ( $P_{app}$ ) across the skin increased with decreasing bromine substitution from BDE-153 ( $P_{app} = 4.0 \times 10^{-4}$  cm/h) to BDE-1 ( $P_{app} = 1.1 \times 10^{-2}$  cm/h). This was accompanied by an increase in the time required to traverse the skin tissue into the receptor fluid (lag time) from 0.25 h for BDE-1 to 1.26 h for BDE-153.  $P_{app}$  values for the studied PBDEs were correlated significantly ( $P < 0.05$ ) with physicochemical parameters like water solubility and  $\log K_{OW}$ . While less brominated congeners achieved faster dermal penetration, higher PBDEs displayed greater accumulation within the skin tissue. The PBDEs thus accumulated represent a contaminant depot from which they may be slowly released to the systemic circulation over a prolonged period. Maximal percutaneous penetration was observed for BDE-1 (~30% of the applied 500 ng/cm<sup>2</sup> dose). Interestingly, BDE-183 and BDE-209 showed very low dermal absorption, exemplified by a failure to reach the steady state within the 24 h exposure period that was studied.



## INTRODUCTION

Polybrominated diphenyl ethers (PBDEs) have found extensive use worldwide as flame retardants used in a wide variety of commercial, domestic, and industrial applications. Applications of PBDEs include electrical and electronic equipment [e.g., TVs, PCs, small domestic appliances (SDAs), etc.] in addition to soft furnishings (e.g., sofas, mattresses, curtains, pillows, etc.).<sup>1</sup> Three technical PBDE formulations were commercially available: Penta (consisting primarily of BDE-47 and BDE-99, 38–49% each, alongside smaller amounts of other tri- to hepta-BDEs), Octa (a mixture of hexa- to deca-BDEs, the exact congener composition varying substantially between the two principal formulations marketed), and Deca (92–97% deca-bromodiphenyl ether, BDE-209, with nona- and octa-BDEs).<sup>2</sup> Deca-BDE has dominated worldwide production with a global market demand of 56100 tons in 2001, compared to 7500 and 3790 tons for Penta-BDE and Octa-BDE formulations, respectively.<sup>1</sup>

Because PBDEs are blended physically within rather than bound chemically to polymeric materials, they migrate from products, after which their persistence and bioaccumulative properties lead to contamination of the environment, including humans.<sup>3</sup> This is of concern because of their potential environmental and toxicological risks, including endocrine disruption, neurodevelopmental and behavioral disorders, hepatic abnormalities, and possibly cancer.<sup>4–6</sup> Moreover, the

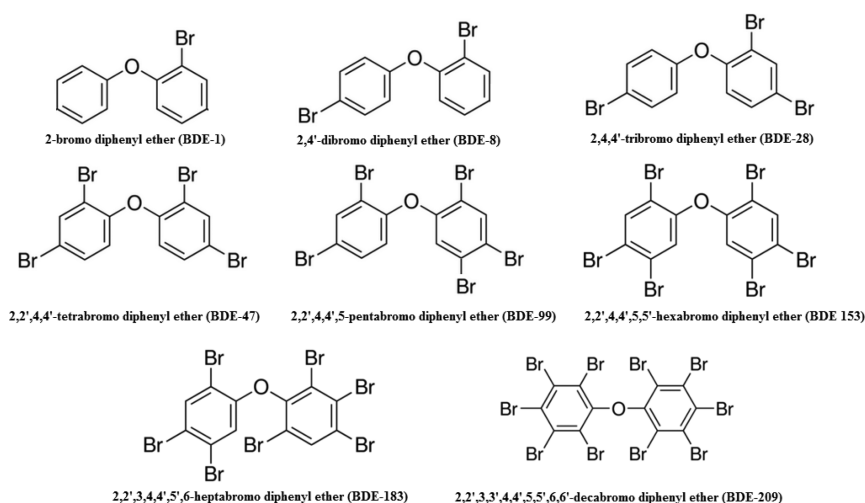
few data available from human epidemiological studies imply effects on male reproductive hormones,<sup>7,8</sup> semen quality,<sup>9</sup> thyroid hormone homeostasis,<sup>10</sup> cryptorchidism,<sup>11</sup> hormone levels and fecundability in adult women,<sup>12</sup> and lower birth weight and length.<sup>13,14</sup> Such evidence has contributed to complete EU bans for the Penta- and Octa-BDE formulations and restrictions on the use of Deca-BDE.<sup>15</sup> In addition, PBDEs associated with Penta- and Octa-BDE are listed under the UNEP Stockholm Convention on persistent organic pollutants (POPs), while Deca-BDE is currently under consideration for listing under Annexes A, B, and/or C of the convention.<sup>16</sup> Despite such restrictions, human exposure to PBDEs is likely to continue for the foreseeable future, given their persistence and the ubiquity of flame-retarded consumer materials.<sup>17</sup>

The current understanding is that nonoccupational human exposure to POPs occurs mainly via a combination of diet, ingestion of indoor dust, dermal contact with dust/consumer products, and inhalation of indoor air.<sup>18</sup> While ingestion of indoor dust is considered the major exposure pathway for many individuals, especially for young children and toddlers,<sup>19</sup> dermal exposure (via contact with indoor dust and flame-retarded products) was predicted to be the second most important

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**Figure 1.** Chemical structure and nomenclature of PBDEs used in this study.

contributor to PBDE body burdens of adult Americans.<sup>20</sup> In Europe, while food was reported as the dominant pathway of human exposure to PBDEs, dermal exposure to dust was ranked in the upper quantiles of exposure routes contributing to human body burdens with either food or dust ingestion.<sup>18</sup> The significance of the dermal route as a pathway of human exposure to PBDEs was further highlighted by Watkins et al., who reported a significant correlation between PBDE levels on hand wipes (assumed to result from contact with contaminated dust or flame-retarded products) and PBDE concentrations in serum from American adults. Moreover, concentrations of PBDEs in indoor dust were strongly correlated with those in hand wipes, and infrequent hand-washers had 3.3 times greater levels of PBDEs in their handwipes than did frequent hand-washers.<sup>21</sup>

Despite the potentially important contribution of dermal uptake as a pathway of human exposure to PBDEs, very little is known about the fraction of PBDEs that becomes bioavailable to humans (i.e., reaches the systemic circulation) following dermal contact. Such a lack of experimental data on the absorbed fractions of various PBDEs following human dermal exposure represents a research gap, which hinders accurate risk assessment of these hazardous chemicals. Furthermore, while it is known that less brominated PBDEs display toxicological effects stronger than those of more brominated congeners,<sup>22</sup> no experimental data about how the degree of bromination affects the human dermal bioavailability of PBDEs exist. Efforts to address this research gap are impeded by several difficulties, including ethical and technical issues inherent to studies involving human tissues, increasing restrictions on the use of laboratory animals in toxicological studies, and the substantial uncertainties associated with extrapolating data from animal studies to humans because of interspecies variation (e.g., skin barrier function, hair follicles, intercellular subcutaneous lipids, etc.).<sup>23</sup>

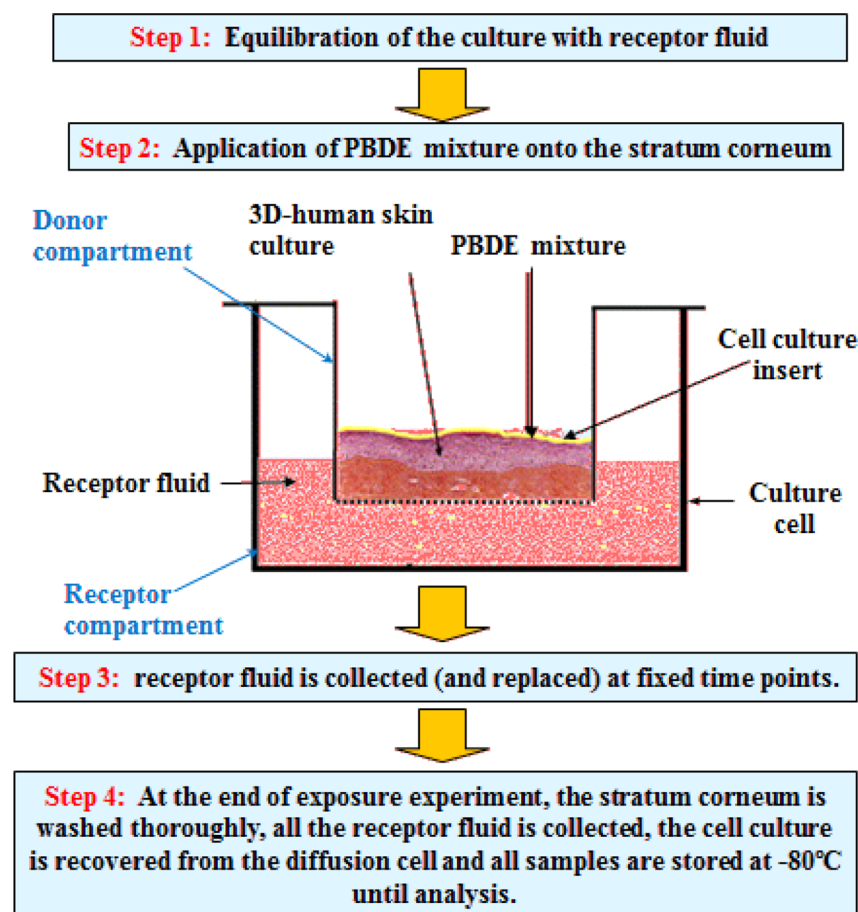
To overcome these difficulties, this study applies *in vitro* three-dimensional human skin equivalents (3D-HSE) as an alternative method to animal and human testing for the assessment of dermal uptake of selected PBDEs. 3D-HSE are commercially available, fully differentiated, multilayer dermal tissues that closely mimic the original human skin both histologically and physiologically.<sup>24</sup> Consequently, validated protocols using 3D-HSE models have been approved by the

OECD (Organisation for Economic Co-operation and Development) and ECVAM (European Centre for Validation of Alternative Methods) for testing skin irritation, phototoxicity, and corrosion by xenobiotic chemicals.<sup>25,26</sup> While recent advances in 3D-HSE culture techniques have resulted in improving their barrier function (up to 85%), it should be noted that no artificial skin model has managed to reach 100% of the barrier function of normal human skin so far. The advantages and limitations of using *in vitro* 3D-HSE to study the percutaneous penetration of various chemicals, including organic FRs, have been comprehensively discussed elsewhere.<sup>23,27</sup> 3D-HSE have been applied within the cosmetics and pharmaceutical sectors to study dermal uptake of various chemicals.<sup>22</sup> Moreover, we recently validated their application to study human dermal absorption of hexabromocyclododecanes (HBCDDs) and tetrabromobisphenol-A (TBBP-A) against human *ex vivo* skin.<sup>28</sup> The specific objectives of this paper are (a) to assess percutaneous penetration of target PBDEs in humans using an EPISKIN 3D-HSE model,<sup>29</sup> (b) to evaluate the influence of bromine substitution on the dermal bioavailability of PBDEs, and (c) to provide the first insights into the dermal bioavailability of several PBDEs.

## MATERIALS AND METHODS

All experiments were performed in a fashion that complied with the principles of good laboratory practice and the OECD guidelines for *in vitro* dermal absorption testing.<sup>29</sup> The handling instructions and performance characteristics of the EPISKIN human skin equivalent model were also taken into consideration. The study protocol received the required ethical approval (ERN\_12-1502) from the University of Birmingham's Medical, Engineering and Mathematics Ethical Review Committee.

**Human Skin Equivalents.** EPISKIN RHE/L/13 human skin equivalent kits were purchased from SkinEthic Laboratories (Lyon, France). The RHE/L/13 tissue constructs are 1.07 cm<sup>2</sup> human skin equivalents resembling the normal human epidermis (Figure SI-1) histologically and physiologically ([www.episkin.com](http://www.episkin.com)). The kit includes maintenance medium (MM), which is a proprietary medium (DMEM, Dulbecco's modified Eagle's medium), that allows acceptable differentiated morphology of the tissue for ~5 days upon receipt by end users. Upon receipt, the EPISKIN tissues were equilibrated



**Figure 2.** General outline of the experimental protocol applied to study the percutaneous permeation of PBDEs.

overnight with the suppliers' MM at 5% CO<sub>2</sub> and 37 °C before use in permeation experiments.

**Chemicals and Standards.** Standard solutions (50 µg/mL, >99% pure) of target PBDE-1 (mono-BDE), PBDE-8 (di-BDE), PBDE-28 (tri-BDE), PBDE-47 (tetra-BDE), PBDE-99 (penta-BDE), PBDE-153 (hexa-BDE), PBDE-183 (hepta-BDE), and PBDE-209 (deca-BDE) (Figure 1) were purchased from Wellington Laboratories (Guelph, ON). PBDE congeners 77 and 128 and [<sup>13</sup>C]BDE-209 used as internal (surrogate) standards and PCB-129 used as a recovery determination (syringe) standard were purchased from the same company. All solvents and reagents used for extraction, cleanup, and instrumental analysis of samples were of the highest available purity and were obtained from Fisher Scientific (Loughborough, U.K.).

**Dosing Solutions.** Two different concentration levels of (I) 5 ng/µL and (II) 10 ng/µL of each of the target PBDEs were prepared in acetone. On the basis of the exposed surface area, net doses of 500 and 1000 ng/cm<sup>2</sup> were applied (infinite dose scenario) to each of the investigated skin tissues using an appropriate volume (100 µL/cm<sup>2</sup>) of dosing solutions I and II, respectively. Acetone was selected as the dosing vehicle on the basis of its ability to dissolve the test compounds at the desired levels and its minimal effect on skin barrier functions. A previous study of the effect of organic solvents on the trans-epidermal water loss (TEWL) as an indicator of skin barrier revealed both acetone and hexane not to exhibit behavior significantly different in this context from that of water, while a

mixture of chloroform and methanol [2:1 (v/v)] caused the most significant increase in TEWL.<sup>30</sup>

**Permeation Assay Protocol.** The permeation experiments were performed using a static configuration (Figure 2). On the basis of the recommendation of the 3D-HSE providers, the EPISKIN tissues were mounted in specific Franz-type permeation devices constructed specifically for this model (SkinEthic Laboratories, Lyon, France) with the *stratum corneum* facing up. All experiments were performed in triplicate. Following a 30 min equilibration, the tested chemicals were applied onto the skin surface in the donor compartment. A DMEM-based culture medium comprising several inorganic salts, vitamins, amino acids, and nutrients (Table SI-1) was used as the receptor fluid, maintained at 32 ± 1 °C, and magnetically stirred; 5% bovine serum albumin (BSA) was added to the receptor fluid to enhance the solubility of target analytes, while the levels of test compounds in the donor solutions were chosen to ensure that the concentrations in the receptor fluid during the experiment did not exceed 10% of the saturation solubility (more details in the Supporting Information).

At fixed time points, aliquots of the receptor fluid (2 mL) were collected from the receptor compartment and immediately replaced with fresh fluid. After 24 h, the entire receptor fluid was collected and the skin surface washed thoroughly with cotton buds impregnated in the 1:1 (v/v) hexane/ethyl acetate solution (five times). The tissues were removed from the permeation devices, and both the donor and receptor compartments were washed separately (5 × 2 mL) with the



**Table 1.** Cumulative Levels (expressed as the average percentage  $\pm$  the standard deviation of the applied dose) of Target PBDEs in the Receptor Fluid following Exposure of EPISKIN to 500 ng/cm<sup>2</sup> of Target PBDEs

time (h)	BDE-1	BDE-8	BDE-28	BDE-47	BDE-99	BDE-153	BDE-183	BDE-209
0.25	ND <sup>a</sup>	ND	ND	ND	ND	ND	ND	ND
0.50	0.25 $\pm$ 0.09	0.10 $\pm$ 0.04	0.07 $\pm$ 0.03	0.04 $\pm$ 0.01	ND	ND	ND	ND
1.00	1.06 $\pm$ 0.28	0.41 $\pm$ 0.05	0.20 $\pm$ 0.02	0.13 $\pm$ 0.02	0.08 $\pm$ 0.01	0.03 $\pm$ 0.01	ND	ND
2.00	1.98 $\pm$ 0.61	0.82 $\pm$ 0.07	0.31 $\pm$ 0.09	0.21 $\pm$ 0.09	0.07 $\pm$ 0.04	0.04 $\pm$ 0.01	ND	ND
6.00	5.07 $\pm$ 1.07	1.88 $\pm$ 0.65	0.46 $\pm$ 0.31	0.48 $\pm$ 0.32	0.43 $\pm$ 0.15	0.13 $\pm$ 0.05	ND	ND
10.00	10.20 $\pm$ 1.89	4.34 $\pm$ 1.72	1.59 $\pm$ 1.56	0.97 $\pm$ 0.82	0.57 $\pm$ 0.77	0.33 $\pm$ 0.03	ND	ND
12.00	14.24 $\pm$ 2.12	6.75 $\pm$ 2.24	2.19 $\pm$ 1.36	1.56 $\pm$ 0.89	0.86 $\pm$ 0.84	0.46 $\pm$ 0.02	0.03 $\pm$ 0.01	ND
18.00	20.43 $\pm$ 2.54	8.68 $\pm$ 2.17	2.77 $\pm$ 1.43	2.23 $\pm$ 0.88	1.29 $\pm$ 1.07	0.68 $\pm$ 0.04	0.04 $\pm$ 0.01	ND
24.00	24.92 $\pm$ 2.71	11.08 $\pm$ 2.43	4.23 $\pm$ 1.68	2.85 $\pm$ 1.09	1.96 $\pm$ 1.26	0.89 $\pm$ 0.11	0.05 $\pm$ 0.01	ND

<sup>a</sup>Not detected (<0.02% of the applied dose for all congeners or 0.05% for BDE-209).

1:1 (v/v) hexane/ethyl acetate mixture. All samples were stored at  $-20^{\circ}\text{C}$  until chemical analysis.

**Sample Extraction and Chemical Analysis.** Each permeation assay generated five different types of samples comprising receptor fluid at various time points, skin tissue, cotton buds (used to thoroughly wipe the skin surface), the donor compartment wash, and the receptor compartment wash. The receptor fluid, skin tissue, and cotton bud samples were extracted according to a previously reported QuEChER-based method<sup>31</sup> (more details are provided as [Supporting Information](#)).

The donor and receptor compartment washes were spiked with 30 ng of the internal standard mixture (BDE-77, BDE-128, and [<sup>13</sup>C]BDE-209) prior to direct evaporation under a gentle stream of N<sub>2</sub>. Target analytes were reconstituted in 100  $\mu\text{L}$  of isooctane containing 100 pg/ $\mu\text{L}$  PCB-129 used as a recovery determination (syringe) standard for quality assurance/quality control (QA/QC) purposes.

Quantification of target PBDEs was performed using a TRACE 1310 GC instrument coupled to an ISQ single-quadrupole mass spectrometer (Thermo Fisher Scientific, Austin, TX) operated in negative chemical ionization (NCI) mode using methane as the reagent gas according to a previously described method.<sup>32</sup> Separation of target PBDEs was performed on an Agilent DB-5 capillary column (15 m  $\times$  0.25 mm, 0.1  $\mu\text{m}$ ) using helium as the carrier gas. The mass spectrometer was run in selected ion monitoring (SIM) mode with ion source, quadrupole, and mass transfer line temperatures set at 230, 150, and 300  $^{\circ}\text{C}$ , respectively. Further details of the GC–NCI/MS method are provided as [Supporting Information](#).

**Data Analysis and Statistical Methods.** A quantitative description of test compound permeation through the skin barrier can be derived from Fick's first law of diffusion as follows:<sup>33</sup>

$$J_{ss} = \frac{\Delta m}{\Delta t A} = \frac{DK\Delta C}{\Delta x} \quad (1)$$

where  $J_{ss}$  is the steady-state flux (nanograms per square centimeter per hour),  $\Delta m$  is the permeated mass (nanograms),  $\Delta t$  is the time interval (hours),  $D$  is the diffusion coefficient (square centimeters per hour),  $K$  is the partition coefficient,  $A$  is the area (square centimeters),  $\Delta c$  is the concentration difference across the membrane (nanograms per cubic centimeter), and  $\Delta x$  is the thickness of the membrane (centimeters).

When using infinite-dose configurations, i.e., in which the donor concentration far exceeds the concentration in the

receptor compartment ( $C_D \gg C_A$ ),  $\Delta C$  can be replaced by the known donor concentration,  $C_D$ , and the permeated mass per time is assumed to be constant. Therefore, the apparent permeation coefficient ( $P_{app}$ , in centimeters per hour), which represents an independent measure of the membrane resistance against permeation of the examined substance, can be calculated as

$$P_{app} = \frac{J_{ss}}{C_D} \quad (2)$$

For each permeation experiment, cumulative amounts of the permeated compounds in the receptor fluid per unit area of exposed skin (nanograms per square centimeter) were plotted versus time (hours). Steady-state conditions were indicated by a linear regression line [ $R^2 \geq 0.9$ ;  $P \leq 0.01$  ([Table SI-6](#))], the slope of which represents the flux ( $J_{ss}$ ). Determination of the start and upper boundary of the linear range (i.e., steady-state conditions) was achieved according to the method previously described by Niedorf et al.<sup>33</sup> (a summary flowchart is provided in [Figure SI-3](#)).

Results are presented as the arithmetic mean of three replicates  $\pm$  the standard deviation (SD). Statistical analysis was performed using SPSS 13.0. Differences in skin permeation were evaluated by the paired Student's  $t$  test between two data sets. A Games–Howell test was used for analysis of variance (ANOVA) among several data sets with equal variances not assumed;  $p < 0.05$  was regarded to indicate a statistically significant difference.

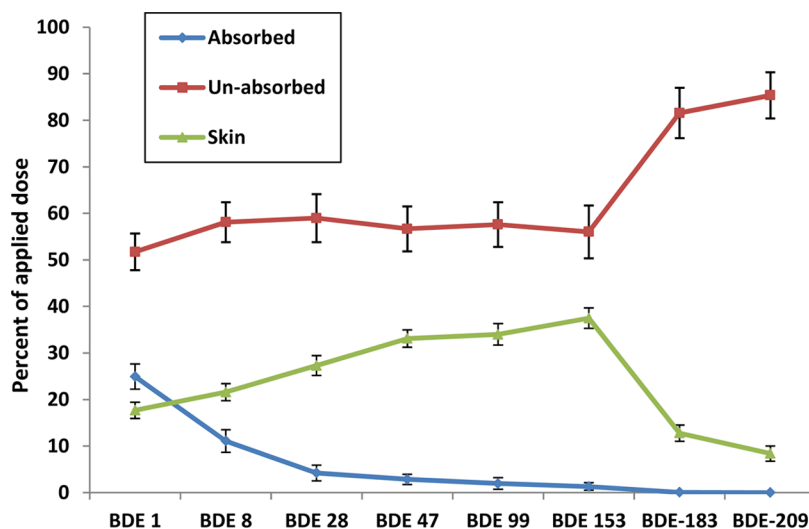
**QA/QC.** Several forms of QA/QC measurement were performed to check the performance of our permeation assay protocol. A “field” blank, comprising a skin tissue exposed to solvents only and treated as a sample, was performed with each sample batch ( $n = 5$ ). None of the studied compounds were above the limit of detection (LOD) in these field blank samples. Good recoveries of internal standards (>80%) were obtained for all samples, indicating the high efficiency of the extraction method ([Table SI-2](#)). The accuracy and precision of the analytical method were tested via replicate analysis of NIST SRM 2585 with certified values for trideca-BDEs. Furthermore, method performance under the applied experimental conditions was tested via matrix spikes of the EPISKIN tissues at three different concentration levels of the target PBDEs. Good results were obtained ([Table SI-3](#)), indicating the suitability of the applied analytical protocol for quantification of target PBDEs in the studied samples.

On the basis of the guidelines of the EPISKIN model, the viability of the tissue was tested by the MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] assay

**Table 2.** Distribution of Target BFRs (expressed as the average percentage  $\pm$  the standard deviation of exposure dose) in Different Fractions of the *in Vitro* Diffusion System following the 24 h Exposure to 500 ng/cm<sup>2</sup> of the Studied PBDEs

	BDE-1	BDE-8	BDE-28	BDE-47	BDE-99	BDE-153	BDE-183	BDE-209
absorbed <sup>a</sup>	24.92 $\pm$ 2.71	11.08 $\pm$ 2.43	4.23 $\pm$ 1.68	2.85 $\pm$ 1.09	1.96 $\pm$ 1.26	0.89 $\pm$ 0.11	0.05 $\pm$ 0.01	ND <sup>c</sup>
unabsorbed <sup>b</sup>	51.72 $\pm$ 3.93	58.11 $\pm$ 4.28	58.97 $\pm$ 5.16	56.66 $\pm$ 4.81	57.61 $\pm$ 4.78	56.02 $\pm$ 5.67	81.57 $\pm$ 5.41	85.35 $\pm$ 4.95
skin	17.69 $\pm$ 1.75	21.60 $\pm$ 1.83	27.29 $\pm$ 2.14	33.10 $\pm$ 1.87	33.98 $\pm$ 2.31	37.49 $\pm$ 2.18	12.78 $\pm$ 1.24	8.38 $\pm$ 1.31
sum	94.33 $\pm$ 7.52	90.79 $\pm$ 8.31	90.49 $\pm$ 8.91	92.61 $\pm$ 7.68	93.54 $\pm$ 8.27	94.39 $\pm$ 7.90	94.40 $\pm$ 6.62	93.73 $\pm$ 6.22

<sup>a</sup>Comprises cumulative concentrations in the receptor fluid over 24 h + receptor compartment rinse. <sup>b</sup>Comprises concentrations in the skin surface wipes after 24 h + donor compartment rinse. <sup>c</sup>Not detected (<0.02% of applied dose for all congeners or 0.05% for BDE-209).

**Figure 3.** Percent of applied dose (500 ng/cm<sup>2</sup>) of target PBDEs absorbed (present in the receptor compartment), unabsorbed (remaining in the donor compartment and on skin surface), and accumulated in the skin tissue following the 24 h exposure.

using a standard kit purchased from the tissue provider. Acceptable MTT results (i.e., Formazan concentration of  $\geq 1.5$  mg/mL) were achieved until 24 h of exposure under the specified test conditions, prior to dropping below the recommended level of Formazan at longer times. Therefore, exposure of EPISKIN tissues to the test PBDE mixtures was terminated after 24 h.

Both positive and negative control experiments were conducted alongside each sample batch. Positive controls involved the exposure of the test tissue to Triton X-100 that showed  $\sim 100\%$  permeation ( $n = 5$ ;  $97 \pm 4\%$ ), while negative controls showed 0% penetration of decabromodiphenyl ethane after exposure for 24 h. The integrity of the skin membrane was tested using visual microscopic inspection and the standard methylene blue (BLUE) method.<sup>34</sup> All EPISKIN tissues used in this study passed all the QA/QC tests mentioned above.

## RESULTS AND DISCUSSION

**Percutaneous Penetration.** The studied PBDE congeners displayed wide variability in their ability to penetrate the skin under the applied experimental conditions (Table 1). Results revealed that the degree of dermal penetration was inversely proportional to the degree of PBDE bromination. Maximal penetration was observed for BDE-1 with  $\sim 30\%$  of the applied dose detected in the receptor fluid after exposure for 24 h, while the more environmentally abundant BDE-47 and BDE-99 showed an average absorption of  $\sim 3$  and  $2\%$ , respectively (Table 1). Interestingly, BDE-209 was not detected in the receptor fluid after 24 h, indicating low dermal bioavailability of this congener in humans (Table 1). Under the infinite-dose configuration applied in this study, the concentration gradient

is maximized and diffusion/penetration through the skin becomes the rate-limiting step. Therefore, the absorbed percentages are likely a function of the applied dose and can vary with the concentration of xenobiotic applied to the skin.<sup>29</sup> However, we have opted to present our results as percentages of the specified dose (Table 1) to facilitate comparison of percutaneous penetration among the various PBDE congeners studied under the applied test protocol.

Results of the mass balance studies were expressed as falling into three major compartments: (1) the directly absorbed dose (cumulative concentration in the receptor fluid over 24 h + receptor compartment rinse), (2) the skin (concentration in the skin tissue after 24 h), and (3) the unabsorbed dose (concentration in the skin surface wipes after 24 h + donor compartment rinse) (Table 2). Data for compartment category (2) revealed all target PBDEs accumulate in the skin tissue to varying degrees. While the proportion of the applied PBDE dose that accumulated in the skin increased with increasing bromine substitution from BDE-1 ( $\sim 18\%$ ) to BDE-153 ( $\sim 37\%$ ), this proportion dropped steeply from BDE-183 ( $\sim 13\%$ ) to BDE-209 ( $8\%$ ) (Figure 3). The increased level of accumulation within the skin of mono- through hexa-BDEs is similar to what was reported for polychlorinated biphenyls (PCBs) in male rats. This *in vivo* rat skin model favored the rapid absorption of lower PCBs (mono- and dichlorinated), while higher PCBs (hexachlorinated) penetrated less rapidly but showed a higher level of accumulation in the skin prior to entering the systemic circulation. This was mainly attributed to the physicochemical parameters of the studied PCBs, which allowed the more polar mono-PCBs to penetrate faster through the water-rich viable epidermis. More lipophilic hexa-PCBs

were hypothesized to accumulate for longer in the lipid-rich *stratum corneum* prior to diffusion through the viable epidermis at a slower rate.<sup>35</sup> In addition to the influence of compound-specific physicochemical parameters, we hypothesize that dermal metabolism may also affect the rate of penetration and accumulation of various PBDEs through the skin. Viable epidermal cells possess cytochrome P450 with activities that are ~80% of hepatic activities. Recent studies have shown various PBDEs to be metabolized by cytochrome P450 to more polar metabolites.<sup>36,37</sup> Biotransformation of different PBDEs by viable human epidermal cells can cause disposition of the metabolized congeners at variable rates. This is likely to influence the concentration gradient of each congener across the various layers of skin and have a substantial effect on the overall rate of percutaneous penetration of the studied compound. However, further research is required to investigate the influence of dermal metabolism on the percutaneous penetration of PBDEs. The low dermal accumulation of BDE-183 and BDE-209 may be attributed to their large molecular mass and volume, which may hinder their partitioning to the *stratum corneum* and subsequent absorption by keratinocytes.<sup>38</sup>

An extensive survey of the available literature reveals very few studies of the dermal absorption of PBDEs in animals and/or humans. In one, radiolabeled BDE-209 showed very low percutaneous penetration (<1%) through female mice skin exposed *in vitro* at three concentration levels. Approximately 2–20% of the dose remained in the skin after exposure for 24 h.<sup>39</sup> If we keep in mind the fact that mouse skin has shown a permeability (7–9-fold) to several chemicals much higher than that of human skin,<sup>39</sup> these findings are generally in agreement with our results for BDE-209, where no penetration to the receptor fluid was observed after exposure for 24 h, but ~8% of the dose was detected in the skin tissue (Table 2). While human absorption of BDE-209 via the dermal route appears to be minimal, this may not be the case for other exposure pathways, including dust ingestion and/or diet. The bioavailability of more brominated PBDEs, including BDE-209, to humans is evident from the results of several biomonitoring studies reporting hepta- to deca-BDEs in various tissues, including human milk, blood, placenta, and adipose tissue.<sup>40,41</sup>

Female mice exposed to 1 mg of [<sup>14</sup>C]BDE-47/kg of body weight in acetone applied to a hairless 2 cm<sup>2</sup> skin patch showed ~62% absorption of the administered dose after 5 days, while 15% remained at the site of application.<sup>42</sup> Another *in vitro* study using frozen, nonviable skin reported a 7.6-fold increase in the percentage of [<sup>14</sup>C]BDE-47 absorbed through rat skin compared with human skin. In the latter report, human skin patches were exposed *in vitro* to a single dose of ~10 mg of [<sup>14</sup>C]BDE-47/cm<sup>2</sup>. Results revealed 3.13% of the initial dose was absorbed after exposure for 24 h, while 33% of the applied dose remained in the skin.<sup>43</sup> Despite the use of a single large dose and a 0.9% (w/v) NaCl solution in water as a receptor fluid, the results of Roper et al.<sup>43</sup> are generally in good agreement with our findings for BDE-47 (Table 2).

To the best of our knowledge, this is the first report of human percutaneous penetration of BDE-1, -8, -28, -99, -153, and -183.

**Dermal Flux ( $J_{ss}$ ) and Permeation Coefficients ( $P_{app}$ ).** Steady-state flux ( $J_{ss}$ ) and skin permeation coefficient ( $P_{app}$ ) values were derived for the studied PBDEs (Table 3 and Figures SI-4 and SI-5). It was not possible to estimate either property for BDE-183 and BDE-209 because of their low

**Table 3. Steady-State Flux, Permeation Coefficient, and Lag Time Values Estimated from Exposure of EPISKIN to 500 ng of Target PBDEs/cm<sup>2</sup> for 24 h**

	flux (ng/cm <sup>2</sup> h)	permeation coefficient (cm/h)	lag time (h)
BDE-1	5.45	$1.09 \times 10^{-2}$	0.25
BDE-8	2.42	$4.84 \times 10^{-3}$	0.42
BDE-28	0.88	$1.76 \times 10^{-3}$	0.82
BDE-47	0.63	$1.26 \times 10^{-3}$	0.90
BDE-99	0.40	$8.00 \times 10^{-4}$	1.10
BDE-153	0.20	$4.00 \times 10^{-4}$	1.26

percutaneous penetration and failure to reach steady state within our 24 h exposure period.

Results show a decreased flux across the skin and higher resistance to percutaneous penetration with increasing bromine substitution from mono- to hexa-PBDEs (Table 3). Variation in the degree of Br substitution across mono- to deca-brominated PBDE congeners is accompanied by substantial changes in their key physicochemical parameters, including molecular weight, water solubility,  $K_{OW}$ , and vapor pressure (Table SI-4). The influence of these parameters on the experimentally derived  $P_{app}$  values for PBDEs targeted in this study (Table 3) was investigated. Results revealed a significantly positive correlation ( $P < 0.05$ ) between  $P_{app}$  values of the studied mono- through hexa-BDEs and the water solubility and vapor pressure of these congeners (Table SI-4). A significant negative correlation ( $P < 0.05$ ) was observed between  $P_{app}$  and  $\log K_{OW}$ , as well as the molecular weight of the studied PBDEs. It was not possible to include BDE-183 and BDE-209 in this statistical analysis because of the lack of  $P_{app}$  values for these congeners as a result of their slow penetration where no steady state was achieved within 24 h. However, our results for mono- through hexa-BDEs are in agreement with previous reports for percutaneous penetration of PCBs<sup>35</sup> and are generally in line with Lipinski's rule of five, indicating that an increase in a chemical's molecular weight or  $\log K_{OW}$  or a decrease in its water solubility is a factor that likely induces higher dermal resistance to the penetration of this compound.<sup>38</sup>

Following the application of a test compound to the skin, it needs to partition into the *stratum corneum* and diffuse through the epidermal cells before reaching the receptor fluid. This results in a lag time,  $t_{lag}$ , with nondetectable flux. The  $t_{lag}$  is represented by the time intercept (i.e.,  $x$ -axis intercept) of the regression line over the linear region of the permeation curve (Figures SI-4 and SI-5). Hence,  $t_{lag}$  can be calculated from eq 3:

$$t_{lag} = \frac{b_0}{J_{ss}} \quad (3)$$

where  $b_0$  refers to the  $y$ -axis intercept of the linear regression line and  $J_{ss}$  is the slope.

Estimated lag times (Table 3) for the studied PBDEs varied between 0.25 and 1.26 h for BDE-1 and BDE-153, respectively. This is in accordance with the ability of less brominated congeners to diffuse quickly through the dermal tissue to the receptor fluid, while the more lipophilic, more brominated congeners are likely to accumulate within the *stratum corneum*. The mass of the chemical retained within the skin tissue may form a depot from which prolonged slow release may occur into the receptor fluid (bloodstream). However, dermal absorption is a dynamic process. Therefore, a certain chemical absorbed into the *stratum corneum* will continue to transfer into viable tissue layers. If there is no loss of the chemical present in



the skin by metabolism, irreversible binding, evaporation, or desquamation, then the mass of the chemical, which entered the skin during the exposure period, will eventually become available to the body.<sup>44</sup>

**Implications for Human Exposure.** Our results indicate that following exposure of a unit area of human skin to a mixture of PBDEs with varying degrees of bromination, less brominated congeners achieve comparatively rapid penetration of the systemic circulation (Table 3). In contrast, more brominated congeners penetrate more slowly through the skin layers to the blood. However, these higher PBDEs will achieve higher levels of accumulation within the skin layers (Table 2). This is likely due to the time required for the more lipophilic, higher-molecular weight PBDEs to penetrate from the *stratum corneum* through the aqueous-based viable epidermis prior to reaching the bloodstream.<sup>44</sup> Durrheim et al. established experimentally that skin stripped of its *stratum corneum* is not infinitely permeable; rather, it retains a residual resistance due to the diffusional barrier of the underlying viable tissue. Because the diffusive medium for a given chemical compound in the viable epidermis is essentially aqueous, this layer displays more resistance to the diffusion of highly lipophilic compounds.<sup>45</sup>

We therefore argue that for the purposes of risk assessment, the total mass of a chemical that becomes systemically available over time following exposure should be considered. Indications from our study are that this value is better expressed by the mass of the target compound that has entered the skin, rather than by that which has traversed the skin. This is because the amount of chemical entering the skin exceeds that exiting the skin (Figure SI-6).<sup>44</sup> Furthermore, the mass of chemical accumulated within the skin tissue is likely to form a contaminant depot, which may release the compound slowly to the bloodstream over a prolonged period of time.<sup>46</sup> Moreover, for higher-molecular weight PBDEs such as BDE-209, metabolism of this skin depot may result in exposure to more toxic lower-molecular weight PBDEs that were not present in the matrix to which the external skin barrier was exposed.

Therefore, the results for percutaneous penetration of BDE-183 and BDE-209 in this study should be regarded with caution in the context of exposure assessment. Although the percutaneous penetration for these congeners over 24 h was almost negligible (Table 2), it should be noted that both compounds accumulated within the skin to varying degrees and may slowly reach the systemic circulation over a prolonged period, even after the exposure is terminated.<sup>46</sup> Hence, the results of this *in vitro* study cannot be considered as conclusive evidence that BDE-209 is not bioavailable to humans via the dermal exposure pathway.

## ■ ASSOCIATED CONTENT

### Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.est.5b03904.

Further details of the analytical methodology, QA/QC parameters, and distribution of target BFRs in different compartments of the *in vitro* diffusion system (PDF)

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## Notes

The authors declare no competing financial interest.

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## ■ REFERENCES

- (1) BSEF, Bromine Science and Environmental Forum. 2014 ([www.bsef.com](http://www.bsef.com)).
- (2) La Guardia, M. J.; Hale, R. C.; Harvey, E. Detailed polybrominated diphenyl ether (PBDE) congener composition of the widely used penta-, octa-, and deca-PBDE technical flame-retardant mixtures. *Environ. Sci. Technol.* **2006**, *40* (20), 6247–6254.
- (3) Harrad, S.; de Wit, C. A.; Abdallah, M. A. E.; Bergh, C.; Bjorklund, J. A.; Covaci, A.; Darnerud, P. O.; de Boer, J.; Diamond, M.; Huber, S.; Leonards, P.; Mandalakis, M.; Östman, C.; Haug, L. S.; Thomsen, C.; Webster, T. F. Indoor Contamination with Hexabromocyclododecanes, Polybrominated Diphenyl Ethers, and Perfluoroalkyl Compounds: An Important Exposure Pathway for People? *Environ. Sci. Technol.* **2010**, *44* (9), 3221–3231.
- (4) Cheng, J.; Gu, J.; Ma, J.; Chen, X.; Zhang, M.; Wang, W. Neurobehavioural effects, redox responses and tissue distribution in rat offspring developmental exposure to BDE-99. *Chemosphere* **2009**, *75* (7), 963–8.
- (5) Darnerud, P. O. Brominated flame retardants as possible endocrine disrupters. *Int. J. Androl.* **2008**, *31* (2), 152–160.
- (6) Wikoff, D. S.; Birnbaum, L. Human Health Effects of Brominated Flame Retardants. In *Handbook of Environmental Chemistry*; Eljarrat, E. B. D., Ed.; Springer: Berlin, 2011; Vol. 16, pp 19–53.
- (7) Palace, V.; Park, B.; Pleskach, K.; Gemmill, B.; Tomy, G. Altered thyroxine metabolism in rainbow trout (*Oncorhynchus mykiss*) exposed to hexabromocyclododecane (HBCD). *Chemosphere* **2010**, *80* (2), 165–169.
- (8) Roosens, L.; Cornelis, C.; D'Hollander, W.; Bervoets, L.; Reynders, H.; Van Campenhout, K.; Van Den Heuvel, R.; Neels, H.; Covaci, A. Exposure of the Flemish population to brominated flame retardants: model and risk assessment. *Environ. Int.* **2010**, *36* (4), 368–376.
- (9) Akutsu, K.; Takatori, S.; Nozawa, S.; Yoshiike, M.; Nakazawa, H.; Hayakawa, K.; Makino, T.; Iwamoto, T. Polybrominated diphenyl ethers in human serum and sperm quality. *Bull. Environ. Contam. Toxicol.* **2008**, *80* (4), 345–350.
- (10) Turyk, M. E.; Persky, V. W.; Imm, P.; Knobeloch, L.; Chatterton, R.; Anderson, H. A. Hormone Disruption by PBDEs in Adult Male Sport Fish Consumers. *Environ. Health Persp.* **2008**, *116* (12), 1635–1641.
- (11) Main, K. M.; Kiviranta, H.; Virtanen, H. E.; Sundqvist, E.; Tuomisto, J. T.; Tuomisto, J.; Vartiainen, T.; Skakkabaek, N. E.; Toppari, J. Flame retardants in placenta and breast milk and cryptorchidism in newborn boys. *Environ. Health Perspect.* **2007**, *115* (10), 1519–1526.
- (12) Butke, D. E.; Wolkin, A.; Stapleton, H. M.; Miranda, M. L. Associations between serum levels of polybrominated diphenyl ether (PBDE) flame retardants and environmental and behavioral factors in pregnant women. *J. Exposure Sci. Environ. Epidemiol.* **2013**, *23* (2), 176–182.
- (13) Chao, H. R.; Wang, S. L.; Lee, W. J.; Wang, Y. F.; Papke, O. Levels of polybrominated diphenyl ethers (PBDEs) in breast milk from central Taiwan and their relation to infant birth outcome and maternal menstruation effects. *Environ. Int.* **2007**, *33* (2), 239–45.
- (14) Thomsen, C.; Stigum, H.; Frøshaug, M.; Broadwell, S. L.; Becher, G.; Eggesbø, M. Determinants of brominated flame retardants in breast milk from a large scale Norwegian study. *Environ. Int.* **2010**, *36* (1), 68–74.



- (15) Roberts, S. C.; Macaulay, L. J.; Stapleton, H. M. In Vitro Metabolism of the Brominated Flame Retardants 2-Ethylhexyl-2,3,4,5-Tetrabromobenzoate (TBB) and Bis(2-ethylhexyl) 2,3,4,5-Tetrabromophthalate (TBPH) in Human and Rat Tissues. *Chem. Res. Toxicol.* **2012**, *25* (7), 1435–1441.
- (16) Stockholm convention on POPs, Persistent Organic Pollutants Review Committee (POPRC), 2013 (<http://chm.pops.int/Convention/POPsReviewCommittee/Overview/tabid/2806/Default.aspx>).
- (17) Harrad, S.; Diamond, M. New directions: Exposure to polybrominated diphenyl ethers (PBDEs) and polychlorinated biphenyls (PCBs): Current and future scenarios. *Atmos. Environ.* **2006**, *40* (6), 1187–1188.
- (18) Trudel, D.; Scheringer, M.; von Goetz, N.; Hungerbühler, K. Total Consumer Exposure to Polybrominated Diphenyl Ethers in North America and Europe. *Environ. Sci. Technol.* **2011**, *45* (6), 2391–2397.
- (19) Harrad, S.; Goosey, E.; Desborough, J.; Abdallah, M. A.; Roosens, L.; Covaci, A. Dust from U.K. primary school classrooms and daycare centers: the significance of dust as a pathway of exposure of young U.K. children to brominated flame retardants and polychlorinated biphenyls. *Environ. Sci. Technol.* **2010**, *44* (11), 4198–202.
- (20) Lorber, M. Exposure of Americans to polybrominated diphenyl ethers. *J. Exposure Sci. Environ. Epidemiol.* **2008**, *18* (1), 2–19.
- (21) Watkins, D. J.; McClean, M. D.; Fraser, A. J.; Weinberg, J.; Stapleton, H. M.; Sjödin, A.; Webster, T. F. Exposure to PBDEs in the Office Environment: Evaluating the Relationships Between Dust, Handwipes, and Serum. *Environ. Health Persp.* **2011**, *119* (9), 1247–1252.
- (22) Darnerud, P. O. Toxic effects of brominated flame retardants in man and in wildlife. *Environ. Int.* **2003**, *29* (6), 841–53.
- (23) Abdallah, M. A. E.; Pawar, G.; Harrad, S. Evaluation of in vitro vs. in vivo methods for assessment of dermal absorption of organic flame retardants: A review. *Environ. Int.* **2015**, *74*, 13–22.
- (24) Schaefer-Korting, M.; Bock, U.; Diembeck, W.; Duesing, H.-J.; Gamer, A.; Haltner-Ukomadu, E.; Hoffmann, C.; Kaca, M.; Kamp, H.; Kersen, S.; Kietzmann, M.; Korting, H. C.; Kraechter, H.-U.; Lehr, C.-M.; Liebsch, M.; Mehling, A.; Mueller-Goymann, C.; Netzlaß, F.; Niedorf, F.; Ruebelke, M. K.; Schaefer, U.; Schmidt, E.; Schreiber, S.; Spielmann, H.; Vuia, A.; Weimer, M. The use of reconstructed human epidermis for skin absorption testing: results of the validation study. *Alternatives to laboratory animals: ATLA* **2008**, *36* (2), 161–187.
- (25) Ackermann, K.; Lombardi Borgia, S.; Korting, H. C.; Mewes, K. R.; Schafer-Korting, M. The Phenion full-thickness skin model for percutaneous absorption testing. *Skin Pharmacol Physiol* **2010**, *23* (2), 105–112.
- (26) Buist, H. E.; van Burgsteden, J. A.; Freidig, A. P.; Maas, W. J.; van de Sandt, J. J. New in vitro dermal absorption database and the prediction of dermal absorption under finite conditions for risk assessment purposes. *Regul. Toxicol. Pharmacol.* **2010**, *57* (2–3), 200–9.
- (27) Jakasa, I.; Kezic, S. Evaluation of in-vivo animal and in-vitro models for prediction of dermal absorption in man. *Hum. Exp. Toxicol.* **2008**, *27* (4), 281–288.
- (28) Abdallah, M. A.-E.; Pawar, G.; Harrad, S. Evaluation of 3D-human skin equivalents for assessment of human dermal absorption of some brominated flame retardants. *Environ. Int.* **2015**, *84*, 64–70.
- (29) OECD, Guideline for the testing of chemicals. Skin absorption: In vitro method. Organisation for Economic Cooperation and Development TG 428, 2004.
- (30) Abrams, K.; Harvell, J. D.; Shriner, D.; Wertz, P.; Maibach, H.; Maibach, H. I.; Rehfeld, S. J. Effect of organic-solvents on in-vitro human skin water barrier function. *J. Invest. Dermatol.* **1993**, *101* (4), 609–613.
- (31) Abdallah, M. A.-E.; Zhang, J.; Pawar, G.; Viant, M. R.; Chipman, J. K.; D'Silva, K.; Bromirski, M.; Harrad, S. High-resolution mass spectrometry provides novel insights into products of human metabolism of organophosphate and brominated flame retardants. *Anal. Bioanal. Chem.* **2015**, *407* (7), 1871–1883.
- (32) Roosens, L.; Abdallah, M. A.; Harrad, S.; Neels, H.; Covaci, A. Factors influencing concentrations of polybrominated diphenyl ethers (PBDEs) in students from Antwerp, Belgium. *Environ. Sci. Technol.* **2009**, *43* (10), 3535–41.
- (33) Niedorf, F.; Schmidt, E.; Kietzmann, M. The automated, accurate and reproducible determination of steady-state permeation parameters from percutaneous permeation data. *Alternatives to laboratory animals: ATLA* **2008**, *36* (2), 201–213.
- (34) Guth, K.; Schäfer-Korting, M.; Fabian, E.; Landsiedel, R.; van Ravenzwaay, B. Suitability of skin integrity tests for dermal absorption studies in vitro. *Toxicol. In Vitro* **2015**, *29* (1), 113–123.
- (35) Garner, C. E.; Matthews, H. B. The effect of chlorine substitution on the dermal absorption of polychlorinated biphenyls. *Toxicol. Appl. Pharmacol.* **1998**, *149* (2), 150–158.
- (36) Erratico, C. A.; Szeitz, A.; Bandiera, S. M. Biotransformation of 2,2',4,4'-Tetrabromodiphenyl Ether (BDE-47) by Human Liver Microsomes: Identification of Cytochrome P450 2B6 as the Major Enzyme Involved. *Chem. Res. Toxicol.* **2013**, *26* (5), 721–731.
- (37) Erratico, C. A.; Szeitz, A.; Bandiera, S. M. Oxidative Metabolism of BDE-99 by Human Liver Microsomes: Predominant Role of CYP2B6. *Toxicol. Sci.* **2012**, *129* (2), 280–292.
- (38) Choy, Y. B.; Prausnitz, M. R. The Rule of Five for Non-Oral Routes of Drug Delivery: Ophthalmic, Inhalation and Transdermal. *Pharm. Res.* **2011**, *28* (5), 943–948.
- (39) Hughes, M. F.; Edwards, B. C.; Mitchell, C. T.; Bhooshan, B. In vitro dermal absorption of flame retardant chemicals. *Food Chem. Toxicol.* **2001**, *39* (12), 1263–70.
- (40) Linares, V.; Belles, M.; Domingo, J. L. Human exposure to PBDE and critical evaluation of health hazards. *Arch. Toxicol.* **2015**, *89* (3), 335–356.
- (41) Frederiksen, M.; Vorkamp, K.; Thomsen, M.; Knudsen, L. E. Human internal and external exposure to PBDEs—a review of levels and sources. *Int. J. Hyg. Environ. Health* **2009**, *212* (2), 109–34.
- (42) Staskal, D. F.; Diliberto, J. J.; DeVito, M. J.; Birnbaum, L. S. Toxicokinetics of BDE 47 in female mice: effect of dose, route of exposure, and time. *Toxicol. Sci.* **2004**, *83* (2), 215–223.
- (43) Roper, C. S.; Simpson, A. G.; Madden, S.; Serex, T. L.; Biesemeier, J. A. Absorption of C-14 -tetrabromodiphenyl ether (TeBDE) through human and rat skin in vitro. *Drug Chem. Toxicol.* **2006**, *29* (3), 289–301.
- (44) U.S. Environmental Protection Agency. Dermal Exposure Assessment: Principles and Applications. EPA/600/8-91/011B, 1992 ([http://www.epa.gov/oppt/exposure/presentations/efast/usepa\\_1992d\\_dermalea.pdf](http://www.epa.gov/oppt/exposure/presentations/efast/usepa_1992d_dermalea.pdf)).
- (45) Durrheim, H.; Flynn, G. L.; Higuchi, W. I.; Behl, C. R. Permeation of hairless mouse skin 0.1. experimental methods and comparison with human epidermal permeation by alkanols. *J. Pharm. Sci.* **1980**, *69* (7), 781–786.
- (46) Frasc, H. F.; Dotson, G. S.; Bunge, A. L.; Chen, C.-P.; Cherrie, J. W.; Kasting, G. B.; Kissel, J. C.; Sahmel, J.; Semple, S.; Wilkinson, S. Analysis of finite dose dermal absorption data: Implications for dermal exposure assessment. *J. Exposure Sci. Environ. Epidemiol.* **2014**, *24* (1), 65–73.